

Amendments to the Specification:

Please replace the paragraph on page 5, lines 5-13, with the following paragraph:

Figures 1A, 1B, 1C, and 1D provide a schematic illustrating ORF0657n related polypeptide regions screened for protection in animals, and some different ORF0657n sequences. Figure 1A illustrates a schematic of polypeptides that were tested and found protective (shown by filled in rectangles), polypeptides tested and found not to be protective (shown by open rectangles), and a polypeptide not tested (hatched box). Figure 1B provides a full-length sequence used as a reference for Figure 1A (SEQ ID NO: 2). Figure 1C illustrates SEQ ID NO: 28. SEQ ID NO: 28 contains a carboxyl “His-Tag” (LEHHHHHH; SEQ ID NO: 64). SEQ ID NO: 28 containing a carboxyl His-Tag is also referred to herein as “His-Tag ORF0657n”. Figure 1D illustrates an ORF0657n^{I+} sequence (SEQ ID NO: 42).

Please replace the paragraph on page 24, lines 23-33, with the following paragraph:

E. coli HMS174(DE3) cells (Novagen) were transformed and grown on LB plates containing kanamycin (30 µg/mL); 3 colonies (UnkC-1, UnkC-2 and UnkC-3) were selected for expression testing. Liquid LB (kanamycin) cultures were incubated at 37°C, 250 rpm until the A₆₀₀ was between 0.6 and 1.0 and then induced by the addition of IPTG to a final concentration of 1 mM followed by three hours further incubation. Cultures were harvested by centrifugation at 5000 x g for 5 minutes at 4°C. Cells were resuspended in 500 µL lysis buffer (BUGBUSTER Bug Buster, with protease inhibitors, Novagen). An equal volume of loading buffer (supplemented with β-mercapto ethanol to 5% final volume) was added prior to heating the samples at 70°C for 5 minutes. Extracts were run on Novex 4-20% Tris-Glycine gels and proteins were visualized (COOMASSIE BLUE ~~Coomassie Blue~~ stained) and blotted onto nitrocellulose and probed with anti-HIS6 antibodies (Zymed).

Please replace the paragraph on page 25, line 30 to page 26, line 2, with the following paragraph:

Fractions containing His-Tag ORF0657n (SEQ ID NO: 28) were detected by Coomassie stained SDS-PAGE and pooled. Pooled fractions were filtered through a 0.2 micron filter to remove particulate material, and were applied on a size-exclusion column (Sephacryl SEPHACRYL S-300 26/60 column, Amersham Biosciences) and eluted at 1 mL/min with 10 mM MOPS pH 7.1, 150 mM NaCl. Fractions containing His-Tag ORF0657n were detected by Coomassie stained SDS-PAGE and Western blotting (anti-tetra His Mab, Qiagen). Endotoxin was removed by filtration through a Zeta-Plus™ Biofilter (CUNO). Protein concentration was determined by BCA (Pierce). Purity was determined by densitometry of Coomassie stained gels.

Please replace the paragraph on page 26, lines 5-10, with the following paragraph:

S. aureus was grown on Tryptic Soy Agar (TSA) (Becton Dickinson, Sparks, MD) plates at 37°C overnight. The bacteria were washed from the TSA plates by adding 5 mL of PBS onto a plate and gently resuspending the bacteria with a sterile spreader. The bacterial suspension was spun at 6000 rpm for 20 minutes using a SORVALL RC-5B centrifuge (DuPont Instruments). The pellet was resuspended in 16% glycerol and aliquots were stored frozen at -70°C.

Please replace the paragraph on page 27, lines 21-27, with the following paragraph:

Genomic DNA was obtained from different *S. aureus* clinical isolates. Clinical isolates were added to 3 mL of Difco Tryptic Soy Broth (Becton Dickinson, Sparks, MD) and incubated overnight at 37°C and 150 rpm. The overnight cultures were centrifuged in 1.5 mL Eppendorf tubes at 14,000 rpm for 5 minutes. The broth was decanted and the pellets re-suspended in 500 µL re-suspension buffer (25% sucrose, 10 mM Tris pH 7.5). A 5 µL aliquot of a 2 mg/ml lysostaphin LYSOSTAPHIN (Sigma-Aldrich, St. Louis, MO) solution was added to each resuspended pellet. Suspensions were then incubated at 37°C for 1 hour.

Please replace the paragraph on page 28, lines 3-5, with the following paragraph:

The isolated DNA was used as a template for PCR. The gene was amplified using a forward primer (ORF0657nF, SEQ ID NO: 67) and reverse primer (ORF0657nR, SEQ ID NO: 68). PCR products were sequenced using standard ~~Big Dye~~ BIG DYE protocols.

Please replace the paragraph on page 37, line 33 to page 38, line 5, with the following paragraph:

The *E. coli* lysate was prepared using ~~BUGBUSTER~~ Bugbuster-Protein Extraction Reagent (NOVAGEN, Madison, WI) following the manufacturer's protocol. Proteins were immunodetected by Western blot using a murine monoclonal antibody ("designated "2H2B8") to ORF0657n as primary antibody and goat anti-mouse IgG (H+L) horseradish peroxidase-linked whole antibody (ZYMED LABORATORIES, South San Francisco, CA) as the secondary antibody. Mab 2H2B8 was generated by immunization of mice with purified *E. coli* produced full-length ORF0657n. Mab 2H2B8 was selected by ELISA and was shown to be specific for ORF0657n. The filters were processed using the BIO-RAD HRP Conjugate Substrate Kit.

Please replace the paragraph on page 40, lines 28-32, with the following paragraph:

For analysis of expression of recombinant yeast ORF0657nH region (SEQ ID NO: 3) by Coomassie staining of SDS-PAGE gels, samples were subjected to electrophoresis on 4-15% gradient Tris-HCl gels (BIO-RAD) in 1X Tris glycine SDS buffer (BIO-RAD) under reducing and denaturing conditions. The gels were stained with ~~Bio-Safe~~ BIO-SAFE Coomassie, a Coomassie G250 stain according to the manufacturer's protocol (BIO-RAD).

Please replace the paragraph on page 41, lines 3-10, with the following paragraph:

To produce ORF0657nH region in *E. coli*, the producing culture was grown overnight in LB broth containing 50 µg/mL kanamycin at 37°C. A pET28 encoding SEQ ID NO: 4 with a

carboxyl His-Tag was used to obtain expression of protein. The next day, 500 μ L of overnight culture was used to inoculate 5.0 mL LB broth plus 50 μ g per mL kanamycin. The culture was grown at 37°C for approximately 3 hours to an OD₆₀₀ of 0.6. Expression was induced with 1 mM IPTG for 3.5 hours at 37°C. The cells were harvested and the cell pellet was stored at –80°C. The *E. coli* lysate was prepared using Bugbuster BUGBUSTER Protein Extraction Reagent (NOVAGEN, Madison, WI) following the manufacturer's protocol.

Please replace the paragraph on page 41, lines 16-30, with the following paragraph:

Production of the desired species was obtained in 5X minus leucine medium containing 2% glucose plus 4% galactose. Transformants containing pUS38 of both strains 1260 and 1309 secreted an ~80-kDa protein that comigrated very closely with yeast internally expressed ORF0657nH region (from nucleic acid encoding SEQ ID NO: 3) and *E. coli* expressed ORF0657nH (from nucleic acid encoding SEQ ID NO: 4 with a carboxyl His-Tag) that was detected by Western and Coomassie staining. In a typical experiment, 500 ng of these control lysates and 25 microliters of medium supernatant were subjected to electrophoresis. The detection was specific; the 80-kDa protein was not detected in a supernatant of a transformant containing vector alone by either Western blot or Coomassie staining. The secreted ~80-kDa protein could correspond to mature non-glycosylated ORF0657nH region (SEQ ID NO: 3) or alternatively, it could contain a few glycosyl residues. A higher molecular weight species in the supernatants was detected by the antibody as well as two lower molecular weight proteins, all of which were stained by ~~Coomassie Blue~~ COOMASSIE BLUE. The higher molecular weight species could contain unprocessed leader and /or could be glycosylated. The low MW species are likely to be degradation products.

Please replace the paragraph on page 43, lines 1-4, with the following paragraph:

Multiple transformants were screened for production of ORF0657nH region using the fermentation conditions described in Example 9. The cell lysates were analyzed for the

production of ORF0657nH region by Western blot analysis or by analysis of SDS-PAGE gels stained with COOMASSIE BLUE ~~Coomassie blue~~.

Please replace the paragraph on page 43, lines 14-18, with the following paragraph:

For analysis of expression of recombinant yeast ORF0657nH (encoded by SEQ ID NO: 32) by Coomassie staining of SDS-PAGE gels, samples were subjected to electrophoresis on 4-15% gradient Tris-HCl Criterion gels (BIO-RAD) in 1X Tris glycine SDS buffer (BIO-RAD) under reducing and denaturing conditions. The gels were stained with ~~Bio-Safe~~ BIO-SAFE Coomassie, a Coomassie G250 stain (BIO-RAD) according to the manufacturer's protocol.

Please replace the paragraph on page 43, lines 26-34, with the following paragraph:

To produce ORF0657nH region (SEQ ID NO: 4 plus a carboxyl His-Tag) in *E. coli*, the producing culture was grown overnight in LB broth containing 50 µg/mL kanamycin at 37°C. The next day, 500 µL of overnight culture was used to inoculate 5.0 mL LB broth plus 50 µg per mL kanamycin. The culture was grown at 37°C for approximately 3 hours to an OD₆₀₀ of 0.6. Expression was induced with 1 mM IPTG for 3.5 hours at 37°C. The cells were harvested and the cell pellets were stored at -80°C. The *E. coli* lysate was prepared using ~~Bugbuster~~ BUGBUSTER Protein Extraction Reagent (NOVAGEN, Madison, WI) following the manufacturer's protocol. To estimate protein size, prestained standards between 10 and 250 kDa were run in parallel with the lysates (BIO-RAD).

Please replace the paragraph on page 46, lines 3-12, with the following paragraph:

For comparison purposes, a ORF0657nG region (SEQ ID NO: 44 with a carboxyl His-Tag) was cloned in an *E. coli* expression vector as described in Example 1 and expressed. To produce the ORF0657nG region in *E. coli*, the producing culture was grown overnight in LB broth containing 50 µg/mL kanamycin at 37°C. The next day, 500 µL of overnight culture was used to inoculate 5.0 mL LB broth plus 50 µg per mL kanamycin. The culture was grown at

37°C for approximately 3 hours to an OD₆₀₀ of 0.6. Expression was induced with 1 mM IPTG for 3.5 hours at 37°C. The cells were harvested and the cell pellets were stored at -80°C. The *E. coli* lysate was prepared using Bugbuster-BUGBUSTER Protein Extraction Reagent following the manufacturer's protocol (NOVAGEN, Madison, WI). To estimate protein size, prestained standards between 10 and 250 kDa (BIO-RAD) were run in parallel with the lysates.

Please replace the paragraph on page 50, lines 13-17, with the following paragraph:

The culture was harvested via hollow fiber tangential flow filtration (AMICON H5MP01-43 cartridge) using an AMICON DC-10 harvest skid (MILLIPORE, Billerica, MA). The permeate was discarded and the cells were concentrated, diafiltered with PBS, and collected by centrifugation at 8000 rpm, 4°C for 20 minutes using a ~~Sorvall Evolution~~-SORVALL EVOLUTION RC (SLA-3000 rotor). Cells were stored at -70°C.

Please replace the paragraph on page 51, lines 10-14, with the following paragraph:

The Clarified Lysate was fractionated on a size-exclusion chromatography (SEC) column (Pharmacia HiPrep-HIPREP 26/60 SEPHACRYL Sephaeryl S-300 HR, mobile phase: 0.2 M MOPS, pH 7.0). Fractions were analyzed by SDS-PAGE with Coomassie detection and Western blotting using ORF0657n protein specific anti-serum (raised against full-length ORF0657n, SEQ ID NO: 28) Fractions that contained product were pooled.